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UTILIZATION OF AGRICULTURE WASTES. *PART I.* PRODUCTION OF FUNGAL PROTEIN FROM RICE AND WHEAT STRAWS

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Agricultural waste of rice and wheat straws were studied for the production of protein and biomass. As these wastes have low protein contents an attempt is made to increase the protein and biomass content of these wastes so as to produce a better product for consumption as food. The studies were conducted using various media and various incubation periods. Some inorganic salts and molasses were added to improve the cultivation of fungi. *Aspergillus oryzae* produced the best results due to its rapid growth which minimized the chance of contamination. Seven days incubation gave the most favourable results in both the agricultural wastes. The maximum production of biomass (33.33%) with a protein value of 20% was obtained with 450 g of rice straw in media no. 2 whereas 400 g of wheat straw on 6 litres of medium produced the best results with 20% biomass and a protein value of 20%.

Key words: Agriculture waste, Fungal protein, *Aspergillus oryzae,* Protein rich feed.

Introduction

The growth of human population and food resources has a direct relationship with each other. However the human populations increase in geometric ratio, but the food resources increase slowly. Thus the population suffers due to food shortage. Hence, a need always exists for developing protein enriched food preferably from materials that are not directly consumed by animals and humans. A useable protein can be produced by the fermentation of carbohydrates from agriculture waste which may be substituted for soyabean meal and related products.

Pakistan is an agricultural country. Wheat and rice being the staple foods in Pakistan, they are produced in large quantities resulting in an excessive amount of agriculture straw as waste. These wastes are usually used as feed for farm animals or as a low quality fuel. Due to the low protein content these wastes do not provide the quality food. Their quality can be improved through raising the protein contents by the use of certain fungi. The utilization of this technique would thus have a dual beneficial effect i.e. it would provide a better quality protein enriched non-toxic food to the farm animals (Graham 1971; William 1976; Toyama 1976; Han-1976; Forage 1978; Nigam 1994) and on the other hand would solve the problem of waste disposal.

The protein rich food for the farm animals prepared by the above technology would be of great economic benefit for the country. Since 70% of the population in the country lives in villages, this process is of utmost importance for the rural population. Under a rural technology programme, the process can be designed to prepare the protein rich food from wheat and rice straws on site. This would save the disposal of these wastes incurred during the harvesting process. The fungi can be grown in open tank fermentors. The production can be undertaken batch wise under non-aseptic conditions in villages under some expert supervision. The farmers or villagers can be trained to utilize the wastes effectively. The process would boost the productivity of farm animals through provision of quality protein enriched food,and would solve the problem of waste disposal.

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Material and Methods

Selection of organism. Aspergillus oryzae, Paceilmoyces elegans and *Trichoderma viridae* were studied for the production of fungal protein in unsterilized conditions, using tap water (Roger 1976). Among the three fungi tested, *A. oryzae* was found to be the best; because of its rapid growth the chance of contamination was minimum.

Agricultural waste. Dried rice straws and wheat straws were used for the production of fungal protein. Before use these straws were grinded and their protein values were studied.

In order to enhance the protein value (Vazques 1992; Ferrell 1983) different combinations of wheat and rice straws were used in the media. (Table 1 and 2).

Maintenance of culture. *Aspergillus oryzae* was grown on Czapeck's Dox agar slants at 24° to 25° C for 7 days and then maintained on the same medium.

Cultural conditions. The grinded straws were used along with molasses and different concectrations of inorganic salts and other ingredients to promote the growth of fungi (Table 1 and 2).

Preparation of molasses. Molasses used as carbon source was prepared according to the method of Hussain *et al* (1997).

The above mentioned five media were prepared in large tube and inoculated with 7 days old *Aspergillus oryzae* culture. They were then incubated at room temperature for different lengths of time. Afterward the cultures alongwith straws were dried and the protein values were calculated. In this way the medium as well as incubation period were optimized for the best production of fungal protein.

Results and Discussion

A. oryzae, P. elegans and *T. viridae* were used in these studies. *A. oryzae* was most suitable for the production of biomass and better protein values. All the experiments were carried out under unsterlized conditions, using tap water instead of distilled water.

Different sets of media were tried using rice straw as substrate. From these five media were selected for further

Table 1
Composition of different media using rice straw as substrate

1. Molasses (6% sugar)	200ml/lit.
$(NH_4)_2 SO_4$	12 gm/lit.
$KH_2 PO_4$	1 gm/lit.
Corn steep liquor	50 gm/lit.
Make up to 6 lit. with tap water	
Rice straws	260 gm.
2. Medium same as No.1	
Make up to 6 lit with tap water	
+ Rice straws	450 gm.
3. Molasses	66.5 ml/lit.
$(NH_4)_2 SO_4$	4gm/lit.
$KH_2 PO_4$	03.5 gm/lit.
Corn steep liquor	17 gm/lit.
Make up to 6 lit. with tap water	
Rice straws	450 gm.
4. Molasses	86 ml/lit.
$(NH_4)_2 SO_4$	5 gm/lit.
$KH_2 PO_4$	0.2 gm/lit.
Corn steep liquor	21 gm/lit.
Make up to 6 lit. with tap water	
Rice straws	428 gm.
5. Molasses	200 ml/lit.
$(NH_4)_2 SO_4$	12 gm/lit.
$KH_2 PO_4$	0.5 gm/lit.
Corn steep liquor	17 gm/lit.
Make up to 6 lit. with tap water	
Rice straws	800 gm.

Table 2
Composition of diffrent media using wheat straw as substrate

1. Molasses (6% sugar)	200 ml/lit.
$KH_2 PO_4$	1 gm/lit.
$(NH_4)_2 SO_4$	12 gm/lit.
Corn steep liquor	30 gm/lit.
Make up to 6 lit. with tap water	
Wheat straws.	450 gm.
2. Molasses (6% sugar)	67 ml/lit.
$KH_2 PO_4$	0.5 gm/lit.
$(NH_4)_2 SO_4$	4 gm/lit.
Corn steep liquor	17 gm/lit.
Make up to 6 lit. with tap water	
Wheat straws.	300 gm.
3. Molasses (6% sugar)	67 ml/lit.
$KH_2 PO_4$	4 gm/lit.
$(NH_4)_2 SO_4$	0.35 gm/lit.
Corn steep liquor	17 gm/lit.
Make up to 6 lit. with tap water	
Wheat straws.	450 gm.
4. Medium same as No.1	
Make up to 6 lit. with tap water	
Wheat straws	800 gm.
5. Molasses (6% sugar)	75 ml/lit.
$(NH_4)_2 SO_4$	4gm/lit.
$KH_2 PO_4$	0.2 gm/lit.
Corn steep liquor	18 gm/lit.
Make up to 6 lit. with tap water	
Wheat straws.	400 gm.

work. Media no.2 and no.5 were the most suitable for the production of fungal protein. It was also concluded that large quantity of rice straws did not produce more fungal mass (Confer media no. 2 and no. 5). It was also concluded that incubation period of 7 days was the most suitable for protein values as well as biomass Table 3.

The same fungi were used in the five media with wheat straw as substrate. Biomass was higher in medium no.1 but the protein value was less as compared to medium no.5. It was therefore concluded that medium-5 was the best Table 4. The incubation period of 7 days was suitable for the best protein values and biomass production.

Table 3

Biomass and protein values obtained from rice straws on different media.

Composition of the medium.		Incubation period	Wt.of dried straw with mycelium	Total biomass production (%)	Protein value of dried mycelium with straw (%)
1. Molasses (6% sugar)	200 ml/lit.	5 days	440 gm.	22.22	14
$(NH_4)_2 SO_4$	11 gm/lit.				
$KH_2 PO_4$	1 gm/lit.				
Corn steep liquor	50 gm/lit.				
Make up to 6 lit. with tap water					
Wheat straws	360 gm.				
2. Medium same as No.1 6 lit. of medium		7 days	600 gm.	33.33	20
Rice straws	450 gm.				
3. Molasses (6% sugar)	66.5 ml/lit.	5 days	550 gm.	22.22	15
$(NH_4)_2 SO_4$	4 gm/lit.				
$KH_2 PO_4$	0.35 gm/lit.				
Corn steep liquor	17 gm/lit.				
Make up to 6 lit. with tap water					
Rice straws	450 gm.				
4. Molasses (6% sugar) 86 ml/lit.		5 days	525 gm.	22.66%	16
$(NH_4)_2 SO_4$	5 gm/lit.				
$KH_2 PO_4$	0.2 gm/lit.				
Corn steep liquor	21 gm/lit.				
Make up to 6 lit. with tap water					
Wheat straws.	428 gm.				
5. Molasses (6% sugar)	200 ml/lit.	7 days	900 gm.	12.5	20
$(NH_4)_2 SO_4$	12 gm/lit.				
$KH_2 PO_4$	0.5 gm/lit.				
Corn steep liquor	17 gm/lit.				
Make up to 6 lit. with tap water					
Rice straws.	800 gm.	-	-	-	

Table 4
Biomass and protein values obtained from rice straws on different media

Composition of the medium		Period incubation	Wt.of dried straw with mycelium	Total biomass production (%)	Protein values of dried mycelium with straw (%)
1. Molasses (6% sugar)	200 ml/lit	7 days	550 gm.	22.22	17.5
$(NH_4)_2 SO_4$	12 gm/lit.				
$KH_2 PO_4$	1 gm/lit.				
Corn steep liquor	50 gm/lit.				
Make up to 6 lit.					
Wheat straws	450 gm.				
2. Molasses (6% sugar)	67 ml/lit	5 days	360 gm.	20	15
$(NH_4)_2 SO_4$	4 gm/lit.				
$KH_2 PO_4$	0.5 gm/lit.				
Corn steep liquor	17 gm/lit.				
Make up to 6 lit.					
Rice straws	300 gm.				
3. Molasses (6% sugar)	67 ml/lit.	5 days	500 gm.	11.1	16
$(NH_4)_2 SO_4$	4gm/lit.				
$KH_2 PO_4$	0.35 gm/lit.				
Corn steep liquor	17 gm/lit.				
Make up to 6 lit.					
Wheat straws	450 gm.				
4. Medium same No.1 + 6 lit.		7 days	900 gm.	12.5	20
Wheat straws	8000 gm.				
5. Molasses (6% sugar)	75 ml/lit.	7 days	400 gm.	20	20
$(NH_4)_2 SO_4$	4 gm/lit.				
$KH_2 PO4$	0.2 gm/lit.				
Corn steep liquor					
Sugar.	18 gm/lit.				
Make up to 6 lit.					
Wheat straws	400 gm.	-	-	-	-

It is concluded from the studies that large quantities of straw are not good for the production of biomass and that the rice straw gives better yield of biomass than wheat straw.

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